PHARMACEUTICAL PROSPECTING AND THE POTENTIAL FOR PHARMACEUTICAL CROPS. NATURAL PRODUCT DRUG DISCOVERY AND DEVELOPMENT AT THE UNITED STATES NATIONAL CANCER INSTITUTE[1]

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Abstract

Chemically-complex natural product drugs are not readily synthesized, and large-scale production for clinical and commercial development often involves isolation from the natural source. The rapidly escalating demand for taxol, originally isolated from the bark of *Taxus brevifolia*, has emphasized the need for alternative sources to the wild plant, and the National Cancer Institute (NCI) has developed policies for exploring such sources at the early stages of preclinical development of potential new drugs. The potential for pharmaceutical crop development in the case of several possible anti-AIDS agents will be discussed.

Humans throughout the ages have relied on plants as a source of food, clothing and construction materials, cosmetics, and medicines. With the soaring world population now exceeding 5.5 billion (World Population Data Sheet, 1993), the consumption of plant and other natural resources is escalating at an unprecedented rate. There is an urgent need to conserve these essential resources through the development of sustainable methods of harvesting and environmentally sound cultivation practices.

Sophisticated traditional medicine systems have been in existence for thousands of years in countries such as China (Chang & But, 1986) and India (Kapoor, 1990), and in the New World tropics (King, 1992). These plant-based systems continue to play the major role in the primary health care of about 80% of the world's inhabitants (World Health Organization statistic, Farnsworth et al., 1985). It has been estimated that plant products constituted at least one active ingredient in about 25% of the prescriptions dispensed from community pharmacies in the United States from 1959 to 1982 (Farnsworth et al., 1985). Well-known examples of plant-derived medicinal agents include the antimalarial drug quinine, the analgesics codeine and morphine, the tranquilizer reserpine, and the cardioactive agent digitoxin.

Despite this evidence, pharmaceutical companies in the western, developed world until recently showed little interest in the investigation of plants as sources of potential new drugs. In recent years, however, major companies, such as Glaxo Chemicals, Merck Pharmaceuticals, Monsanto Company, and SmithKline Beecham, have started screening plants for a range of biological activities. The plant samples are generally provided through collections carried out by botanical institutions or intermediary organizations, some of which are located in developing source countries. The development of these collection and screening operations has been accompanied by an increasing awareness of the need to conserve valuable biological resources, and a recognition of the rights

[1] NCI gratefully acknowledges the collaboration and support of the many individuals and organizations worldwide that make these programs possible. NCI recognizes the indispensable contributions being made through the provision of valuable natural resources, expertise, knowledge, and skills; through policies of collaboration and compensation, as stated in the Letter of Intent, NCI wishes to assure participating countries of its intentions to deal with them in a fair and equitable manner.

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of source country organizations and peoples to the accrual of benefits from the development of any new drugs derived from their countries' genetic resources.

The National Cancer Institute Plant Acquisition Program

Historically, many claims have been made for the beneficial use of plants in the treatment of cancer (Hartwell, 1982), though many of these claims may be viewed with some skepticism, since cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine. The development of efficacious anticancer agents, such as the so-called Vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don, and etoposide and teniposide, semisynthetically derived from podophyllotoxin isolated from *Podophyllum peltatum* L. and *P. emodii* Wall. (Cragg et al., 1993b), provided convincing evidence that plants could be sources of a variety of novel cancer chemotherapeutic agents. Prompted by such discoveries, in 1960 the National Cancer Institute (NCI) initiated a systematic program for the collection and screening of plants for antitumor activity. Between 1960 and 1981, the NCI, in collaboration with the United States Department of Agriculture (USDA), collected and tested more than 114,000 extracts of some 35,000 plants against a range of animal tumor systems (Perdue, 1976). While a large number of agents, belonging to a wide variety of chemical classes, were isolated and characterized (Hartwell, 1976; Suffness & Douros, 1979), few satisfied the stringent preclinical development requirements and advanced to human clinical trials. Clinically active agents to emerge from this program included taxol from *Taxus brevifolia* Nutt. and other *Taxus* species (Cragg et al., 1993a), topotecan and CPT-11, semisynthetic derivatives of camptothecin from *Camptotheca acuminata* Decne., and homoharringtonine from *Cephalotaxus harringtonia* var. *drupacea* (Sieb. & Zucc.) Koidzumi (Cragg et al., 1993b).

The NCI–USDA collaborative program concentrated on plant collections in temperate regions. Since 1986, however, the NCI has expanded its program to the collection of plants from tropical and subtropical regions worldwide through the award of contracts to the Missouri Botanical Garden (Africa and Madagascar), the New York Botanical Garden (Central and South America and the Caribbean), and the University of Illinois at Chicago, assisted by the Arnold Arboretum at Harvard University and the Bishop Museum in Honolulu (Southeast Asia). In carrying out these collections in over 20 countries, these NCI contractors work closely with qualified organizations in each of the source countries. Scientists from source country organizations collaborate in field-collecting activities and taxonomic identifications. Their knowledge of local species and conditions has been indispensable to the success of the NCI collection operations to date. Source country organizations provide facilities for the preparation, packaging, and shipment of the samples to the NCI natural products repository in Frederick, Maryland, and their personnel have provided invaluable assistance to the NCI contractors in obtaining the necessary collection and export permits. The collaboration between the source country organizations and the NCI collection contractors has, in turn, provided support for expanded research activities by source country scientists, and the deposition of a voucher specimen of each species collected in the national herbarium is expanding source country holdings of their own flora. In addition, NCI contractors provide training opportunities for local personnel through workshops and lectures.

In expanding the plant collection program to tropical and subtropical regions, the NCI recognized that tropical forests contain well over 50% of the estimated 250,000 plant species found on earth; only a small percentage have been investigated for their chemotherapeutic potential, and relatively few had been screened in the earlier NCI program. In addition, the rapid destruction of tropical forests and the disappearance of indigenous knowledge associated with the use of many of the plants lent an urgency to the initiation of a collection program before these valuable resources had been too severely depleted. While investigating the potential of these plants in drug discovery and development, the NCI also wishes to promote the conservation of biological diversity and recognizes the need to compensate source country organizations and peoples in the event of commercialization of a drug developed from a plant collected within their borders (Cragg et al., 1994).

Extracts of the plants are tested in vitro for selective cytotoxicity against panels of human cancer cell lines representing major disease types including leukemia, melanoma, lung, colon, breast, central nervous system, ovarian, and renal cancers (Boyd, 1989; Grever et al., 1993). With the emergence of the acquired immunodeficiency syndrome (AIDS) as a global epidemic in the 1980s, the NCI was assigned the additional mission of the discovery

and preclinical development of novel anti-HIV agents; since 1988 all plant extracts have also been tested for in vitro anti-AIDS activity in a screen comprising human lymphoblastoid cells infected with the live AIDS virus (Boyd, 1988). Extracts showing significant selective cytotoxicity or anti-AIDS activity are subjected to bioassay-guided fractionation by NCI chemists, and the isolated active agents, after complete structural characterization, are entered into various stages of preclinical development (Cragg et al., 1993b). Satisfactory completion of preclinical studies and approval by the Food and Drug Administration (FDA) clears the drug for advancement to clinical trials and, subject to further FDA approval, to eventual marketing and general clinical use.

Even though the percentage of extracts showing preliminary activity in the in vitro screens might vary between 1% and 5%, the number of potentially valuable "leads" approved for preclinical development is more likely to be one in 5000 to 10,000. Given the stringent requirements for preclinical and clinical approval, the chances of developing an effective commercial anticancer drug against any of the diseases tested for are of the order of one in 50,000 samples screened. The timespan for development can vary considerably, from 10 to 20 years for anticancer drugs, with the cost estimates for discovery and development exceeding $230 million (DiMasi et al., 1991).

Drug Development and Supply Issues: The Potential for Pharmaceutical Crops

The initial plant sample (0.3–1.0 kg) collected by the contractor generally yields enough extract (10–40 g) to permit isolation of the pure, active constituent in sufficient milligram quantity for complete structural elucidation. Subsequent secondary testing and preclinical development, however, might require gram or even kilogram quantities, while approval for clinical development could require multi-kilogram quantities.

To obtain sufficient quantities of an active agent for preclinical development, re-collections of 5 to 200 kg of dried plant material, preferably from the original collection location, might be necessary; considerably larger quantities would be required for subsequent clinical development. Large re-collections necessitate surveys of the distribution and abundance of the plant, as well as determination of the variation of drug content in different plant parts and the fluctuation of content with the season of harvesting. The potential for mass cultivation of the plant would also need to be assessed. If problems are encountered due to scarcity of the wild plant or inability to adapt it to cultivation, a search for alternative sources would be necessary. Other species of the same genus or closely related genera can be analyzed for drug content, and techniques, such as plant tissue culture, can be investigated. While total synthesis must always be considered as a potential route for bulk production of the active agent, it should be noted that the structures of most bioactive natural products are extremely complex, and laboratory bench-scale syntheses often are not readily adapted to large-scale economic production.

Of the established plant-derived commercial anticancer drugs, vinblastine and vincristine are still produced by isolation from *Catharanthus roseus* grown in various regions worldwide, while etoposide and teniposide are semisynthetically produced from natural precursors isolated from *Podophyllum emodii* harvested in India and Pakistan. Over the past 20 to 30 years extensive research has been carried out on the development of synthetic and tissue culture procedures for the production of these drugs, particularly the Vinca alkaloids, but use of the plant sources remains the most economically viable method.

The development of sources for the large-scale production of taxol illustrates the necessity for studying the various methods of biomass production at an early stage of drug development in order to cater to escalating demands (Cragg et al., 1993a). Between 1977 and 1988 supplies for preclinical and early clinical development were easily met by isolation from the bark of the Pacific Yew (*Taxus brevifolia*), the original source of the drug. Up to 1990, approximately 4 kg had been isolated from the bark, but discovery of the efficacy of taxol in the treatment of refractory ovarian cancer in 1988 increased the potential demand to over 24 kg per year in the United States alone. Given the yield at that stage of one gram of taxol from approximately 13 kg of dried bark (equivalent to about 1.5 mature trees), the anticipated demand of over 24 kg required the processing of 312,000 kg from about 36,000 mature trees.

The discovery of taxol's efficacy in the treatment of breast cancer, and observations of preliminary activity against other cancers, such as lung and head-and-neck cancer, created potential demands exceeding 400 kg per year in the United States, and it was clear that alternative sources to the bark would need to be developed. In 1991, the pharmaceutical company Bristol-Myers Squibb (BMS) signed a Cooperative Research and Development Agreement (CRADA) with the NCI whereby it be-

came responsible for the continued production of taxol. Since the bark of *T. brevifolia* remained the only FDA-approved source of taxol, BMS and its partners, Hauser Chemical Research and Hauser Northwest, continued bark collections of over 700,000 kg per year in 1991 and 1992. BMS and the NCI, however, immediately embarked on intensive studies of alternative methods of bulk production and alternative sources of taxol. A key to solving the taxol supply problem was provided by the pioneering studies of French workers, who demonstrated that natural precursors, such as baccatin III and 10-desacetylbaccatin III, can be converted to taxol and related active agents by relatively simple semisynthetic methods (Denis et al., 1988). Extensive analytical surveys of *Taxus* species and cultivars worldwide showed that the needles of many species contain reasonable quantities of the baccatin precursors, thus providing an abundant renewable source of taxol and related agents. In addition, millions of *Taxus* plants, representing a wide range of species and cultivars, are grown in U.S. nurseries for sale as ornamental shrubs. These, together with the mass cultivation of *Taxus* strains containing relatively high yields of taxol or its precursors by companies, such as Weyerhaeuser Company, further expand the renewable needle resource. BMS recently announced that it will be phasing out the use of bark and will no longer be collecting the bark of *T. brevifolia* growing on public, federal land; bark will be replaced by the semisynthetic route from the baccatin precursors as the major source of taxol. The baccatin precursors are currently isolated by the Italian company Indena S.P.A., from needles harvested from *Taxus* species in Europe and India.

Meanwhile, taxol and related compounds have been produced by plant tissue culture, and the scale-up production from this source is being developed by companies such as ESCAgenetics and Phyton Catalytic. The total synthesis of taxol has yet to be completed, but substantial progress has been made, starting from the abundant constituent of pine trees, pinene. While the total synthesis is unlikely to provide an economically competitive alternative to the semisynthetic approach using baccatin precursors, it might provide simpler synthetic intermediates that retain the desired anticancer activities and are more amenable to large-scale synthesis.

With positive responses being seen in the treatment of various cancers in the early clinical trials of the camptothecin derivatives, CPT-11 and topotecan (Eckhardt et al., 1992; Masuda et al., 1992; Takeuchi et al., 1992), it is likely that the demand for camptothecin will escalate. In addition, another camptothecin derivative, 9-amino camptothecin, has recently entered clinical trials. While a reasonable supply of camptothecin is currently available from Chinese and Indian sources, the NCI has acted to preclude any possible shortage of these drugs through initiation of a project for the cultivation of the source tree, *Camptotheca acuminata*, in collaboration with the USDA Forest Service. In this instance, considerable experience has been gained from USDA cultivation projects in the 1960s when camptothecin itself was a clinical candidate (Perdue et al., 1970).

Recent Developments in the NCI Natural Product Drug Discovery and Development Program

POLICIES FOR BIOMASS PRODUCTION

The experience gained in the production of taxol highlighted the necessity for studying various methods of biomass production at an early stage of development of a new anticancer or anti-AIDS agent. To this end, the NCI has implemented a Master Agreement (MA) mechanism whereby pools of qualified organizations have been established with expertise in: the large-scale re-collection of source plant materials; the cultivation of source plants; and source plant tissue culture. The areas of cultivation and tissue culture are divided into two phases, one involving the initiation of pilot-scale studies to explore the feasibility of the techniques for production of the desired agent, and the second involving application of the methods developed in the feasibility studies to large-scale production. When a plant-derived agent is approved for preclinical development, Master Agreement Order (MAO) Requests for Proposals (RFPs) for projects in one or more of the above areas are issued to the relevant pools of MA Holders who then submit technical and cost proposals addressing the particular RFP specifications. An award is made to the MA Holder whose proposal is considered best suited to the Government's needs.

RECENT DISCOVERIES

To date, more than 35,000 plant samples have been collected by NCI contractors, and over 25,000 have been extracted to yield more than 50,000 organic solvent and aqueous extracts. Over 25,000 of these extracts have been tested in the anti-HIV screen, and about 2700 have exhibited some in vitro activity; of these, close to 2400 are aqueous

extracts, and in the majority of cases the activity has been attributed to the presence of ubiquitous chemotypes, such as polysaccharides and tannins. Such compounds are not a current NCI focus for drug development and typically are eliminated early in the discovery process.

A number of novel in vitro active anti-AIDS agents have been isolated and selected for preclinical development. The dimeric alkaloid michellamine B has been isolated from the leaves of the tropical vine *Ancistrocladus korupensis* D. Thomas & Gereau, collected in the rainforest regions of southwestern Cameroon (Manfredi et al., 1991; Thomas & Gereau, 1993). Michellamine B shows in vitro activity against both the HIV-1 and HIV-2 forms of the AIDS virus and is in advanced preclinical development. Preliminary surveys of the occurrence and abundance of *A. korupensis*, as well as cultivation experiments, have been carried out by the Missouri Botanical Garden through an extension of its current contract with the NCI. Surveys thus far indicate that its range and abundance are very limited, but fallen leaves collected from the forest floor have been shown to contain reasonable quantities of michellamine B; the collection of these leaves has obviated the large-scale harvest of fresh leaves and avoided possible endangerment of the wild species. Fallen leaf collections will provide sufficient michellamine B to complete preclinical studies, but the NCI is proceeding with feasibility studies of the cultivation of the plant through the Master Agreement process. The collections and cultivation experiments are being performed with the full participation of Cameroon authorities and scientists, as well as close collaboration with the World Wide Fund for Nature, which is coordinating conservation projects in the Korup region of Cameroon. Thus far, no other *Ancistrocladus* species have shown significant anti-HIV activity.

Calanolide A is a novel coumarin isolated from the leaves and twigs of the tree *Calophyllum lanigerum* Miq. var. *austrocariaceum* (T. C. Whitmore) P. F. Stevens, collected in the rainforest regions of Sarawak, Malaysia (Kashman et al., 1992). Calanolide A shows potent in vitro activity against HIV-1 and several resistant strains of the virus, but not against HIV-2, and is in early preclinical development. Re-collections of plant material identified as *C. lanigerum* from the same general location have shown a range of test results, varying from reasonable activity to total lack of activity. It is apparent that the production of calanolide A is dependent on various factors, possibly including the immediate growth environment of *C. lanigerum* and the time of harvest. Careful taxonomic and chemotaxonomic studies of this species are being performed by the NCI contractor, the University of Illinois at Chicago, in collaboration with the NCI and scientists from Sarawak. A survey of related species has shown that the latex of *C. teysmannii* var. *inophylloide* P. F. Stevens, collected in the same region, yields the related compound costatolide, which has significant in vitro anti-HIV activity, though being somewhat less active than calanolide A. Costatolide, or a suitable derivative, might also be considered for preclinical development. The latex contains high yields of costatolide and would be an excellent renewable source of the compound should it advance to clinical development.

A novel trimeric naphthoquinone derivative, conocurvone, has been isolated from a *Conospermum* species endemic to western Australia (Decosterd et al., 1993); this plant was collected for the NCI program by the USDA in 1981. Conocurvone exhibits potent in vitro activity against HIV-1 and is in early preclinical development. Conocurvone has been synthesized from the monomeric naphthoquinone, teretifolione B, also isolated from the plant, while simpler trimeric naphthoquinone analogs have been synthesized and shown to possess equivalent in vitro anti-HIV activity. The development of conocurvone or related compounds will be undertaken in close collaboration with Australian scientists, and surveys of the occurrence and abundance of the source plant and related *Conospermum* species are being carried out by the Western Australian Department of Conservation and Land Management (CALM).

Another potential anti-AIDS agent, prostratin, has been isolated from the stemwood of the western Samoan tree, *Homalanthus nutans* (Forster) Pax (Gustafson et al., 1992). This tree is used in western Samoa for the treatment of a variety of diseases, including yellow fever (Cox, 1990); an extract of the stemwood was provided by Paul Cox of Brigham Young University, Utah. While prostratin belongs to the phorbol class of compounds, which frequently exhibit significant tumor-promoting properties, it does not appear to be associated with tumor promotion and has been selected for early preclinical development.

Of the approximately 30,000 extracts tested so far in the in vitro human cancer cell line screen, less than 1.0% have shown some degree of selective cytotoxicity. Interesting, novel patterns of differential cytotoxicity have been observed, some of which have been associated with known classes of compounds such as cardenolides, cucurbitacins, lig-

nans, and quassinoids, while others appear to be new leads which are being investigated further.

Conclusion

Plants have played an important role in the NCI drug discovery program for over 30 years, and important plant-derived anticancer agents discovered in this program include taxol and camptothecin, which has been converted to several clinically effective analogs. The NCI also played a significant role in the development of clinically used drugs, such as vinblastine and vincristine, and the podophyllotoxin analogs, etoposide and teniposide. While considerable effort has been devoted to the production of these drugs by alternative methods, such as synthesis and tissue culture, it is significant that the wild or cultivated plants remain the major sources of either the drugs themselves or key precursors. Experience with the large-scale production of taxol has emphasized the need for the investigation of alternative sources to the wild plant at early stages of preclinical development of potential new drugs, and the NCI has introduced policies for achieving this goal. The collection and screening of plants from over 20 countries for anticancer and anti-HIV activity, and the isolation of some novel active agents, presents the prospect for the development of pharmaceutical crops in some of these countries. It must be emphasized, however, that drug discovery and development is a lengthy process requiring 10 to 20 years of intensive research, and very few new agents eventually succeed in reaching the market as effective drugs.

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